Tristeza Survey in the West Indies

C. Bujadoux
M.L. Caruana
Laboratoire de virologie
CIRAD-FLHOR
BP 5035
34032 Montpellier cedex 01
France

Fruits, vol. 49, n°5-6
p. 410-414 (English)
p. 488-490 (French)

A comprehensive indexing operation has been under way since 1993 in experimental orchards of Martinique and neighbouring islands to map the distribution of citrus tristeza virus in the West Indies. Behavioural studies on vectors of this disease have been conducted for potential biological control purposes.

introduction

Citrus cropping is expanding in the West Indies, mainly because of the increased demand for oranges to be processed for juice. Through its research, CIRAD-FLHOR is striving to promote citrus production that is fully adapted to local conditions. In 1988, an experimental orchard was thus set up at Rivière Lézarde (Martinique) to study citrus behaviour in a wet tropical climate and propagate indexed plant material.

However, there is a new citrus disease threat in the region, as signaled by the detection of the brown citrus aphid, *Toxoptera citricidus* Kirkaldy. This is the most efficient vector of citrus tristeza virus (CTV), which is transmitted semi-persistently.

The distribution range of *T. citricidus* recently extended considerably from its endemic zone in the southern hemisphere towards North America (Roistacher *et al.*, 1991).

In Central America, the vector was first noted in Panama in 1985, then in Costa Rica, Salvador and Honduras.

T. citridus colonized the West Indies within 5-6 years; it was first identified in Trinidad and Tobago in 1990, and then in Martinique and Guadeloupe.

In addition, CTV propagated and spread to new areas along with its most efficient vector. A serious infection broke out in Central America in 1992. The Caribbean islands were then contaminated, with severe tristeza strains detected in Trinidad and Tobago, Jamaica, the Dominican Republic and even Cuba.

CTV has a very high destructive potential in citrus orchards of the region since sour orange is the most common rootstock and its associations with orange, mandarin and grapefruit are very susceptible to the virus. The CTV front should thus be carefully monitored.

An indexing operation was set up by CIRAD-FLHOR at Fort-de-France (Martinique) in 1993 to deal with this situation. The operation is focused on trees from SRA lines, which are distributed as virus-free material. Some private citrus orchards in Martinique and hundreds of neighbouring islands have also been surveyed. The overall aim of this operation is to assess the CTV status in Martinique and surrounding islands. This operation has been stepped up in conjunction with the increased brown citrus aphid (*T. citricidus*) populations.

CTV control techniques mainly involve using resistant rootstock, cross protection and eradication. Eradication is used as a preventive strategy if the virus has not yet been propagated. Control programmes have not seriously focused on the insect vectors even though they represent the second most important means of transmission of CTV after grafting.

Studies have been carried out in the Mediterranean region on *Lysiphlebus tes-*

taceipes Cresson (Hym., Braconidae), an endoparasite of many different aphid species (STARY *et al.*, 1988a and 1988b) whose brown citrus aphid biocontrol potential looks promising (YOKOMI, 1992). This endoparasite should therefore be further identified and investigated in full detail.

Studies on the behaviour of *Toxoptera citricidus* and *Lysiphlebus testaceipes* and their interactions are also under way.

materials and methods

phytosanitary situation

plant sampling

Sampling was done at two separate sites in the experimental Citrus orchard at Rivière Lézarde (CIRAD-FLHOR, Martinique). Half of the trees at the first site, which includes 167 different varieties, were tested. A quarter of the trees at the second site, comprising trial plots with replicates of four trees, were sampled. In surveyed private orchards of Martinique and neighbouring islands (Saint Lucia, Saint Vincent, Grenada, Dominica, Antigua, Nevis and Saint Kitts), samples were collected from the most susceptible trees: rootstock/susceptible variety associations, or highly susceptible Citrus trees showing specific symptoms (e.g. vein clearing in lime).

All samples were cut from young lignified shoots.

CTV detection

• ELISA-DAS technique

Indexing was performed using the ELISA-DAS technique with polyclonal antibodies from SANOFI diagnostic kits. The tests were carried out according to the manufacturer's instructions, and the conjugate was depleted with 33 µl of healthy control plant material/ml conjugate solution.

Mauritius papeda (*Citrus hystrix*) was preferentially used as control material for the depletion operation since the virus purified to develop the serum for the diagnostic kit had been obtained from this *Citrus* species. The ELISA plates were read on a spectrophotometer (405 nm) 45 min and 1 h after the substrate was plated.

• analysis of the results

The detection threshold was set 2- or 3-fold higher than the mean optical densities (OD) of the healthy controls (X).

Samples with OD levels lower than 2X were negative (healthy), while those higher than 3X were positive (contaminated). When the OD level was between these two levels, samples were tested a second time before determining the definitive result.

This detection threshold reduced the risks of making diagnostic errors for a disease with 0° tolerance.

insect rearing

aphids

T. citricidus aphids were reared on nursery Mexican lime plants. The plants were cut back regularly to promote the growth of shoots upon which the insect colonies were reared. The aphids had been identified and collected in the field.

Aphids were reared in an insectproof cage under a shaded plastic tunnel in natural conditions: 25-35°C, 12L/12D photoperiod and about 95% relative humidity during the rainy period.

The reared aphids were put in fine cotton sleeves placed around pretreated young shoots in cv Satsuma mandarin (*Citrus unshiu*) trees in the experimental orchard. The progeny of these aphids were then put in separate sleeves.

These aphid were observed on a daily basis to determine their characteristics and behaviour under the environmental conditions of Martinique.

parasitoids

The *L. testaceipes* aphid endoparasites were reared under the same conditions as the aphids on Mexican lime branches collected from the *T. citricidus* rearing cages; these branches were replaced regularly. Leaves with parasitized aphids (brown and mummy-like) were put in rearing boxes and placed in a room at constant temperature (25°C) under a 12L/12D photoperiod.

L. testaceipes insects hatching on the same day were grouped to determine the length of their adult life cycle.

Two-day-old *L. testaceipes* adults from the rearing boxes were put in the cotton sleeves containing aphids of different ages (3-10 days old) and densities (10-30 aphids).

These parasitoids were removed after 24 h with the host. After 4 days, they were monitored daily to assess the length of their growth cycle and parasitism rates.

The fine cotton sleeves were placed on cv Navel orange trees.

results and discussion

indexing

None of the 665 samples from Martinique were found to be contaminated with CTV (Table 1).

These results confirm the good health status of the experimental orchard at Rivière Lézarde (CIRAD-FLHOR, Martinique) where many samples had been collected. The station can thus continue its plant propagation activities under close supervision.

No general conclusions can be drawn from the present results because of the very low number of samples collected from *Citrus* growers' orchards (two were surveyed); they only apply to the trees tested.

Table 1
Results of CTV indexing analyses using the ELISA-DAS technique.

Origin	Total trees	Trees tested	Healthy trees	Infested trees
Martinique				
Repository orchard	838	420	420	0
Trial plots	956	220	420	0
Plantations	18 250 [1]	25	25	0
Caribbean				
Saint Lucia		10	9	1
Saint Vincent		11	11	0
Grenada		8	8	0
Dominica [2]		18	18	0
Antigua		13	12	1
Nevis		11	11	0
Saint Kitts		7	7	0

(1) estimated
(2) no samples were found to be positive, despite observed CTV symptoms in the field.

Martinique still seems to be CTV-free, but the survey carried out in the West Indies revealed the presence of the virus on two of the seven monitored islands, i.e. Antigua and, closer to Martinique, Saint Lucia. Table 2 illustrates CTV propagation patterns in the West Indies, which are closely correlated with the vector distributions.

Although the sample sizes were small for these areas, CTV seemed to be widespread because of the broad distribution range of its vector. It seems that the introduction of CTV-infected plants was responsible for spreading the virus to the West Indies; it was then transmitted by *T. citricidus*.

This hypothesis is supported by the fact that in Martinique, where only SRA-type citrus material is present (originating from the repository at San Giulano, Corsica, an island that is also virus-free), all ELISA results were negative.

In contrast, CTV has been introduced on neighbouring islands through budwood or plants originating from Florida, a region that has long been known to harbour tristeza.

The arrival of *T. citricidus* should accelerate CTV transmission on islands that until now have only been partially contaminated, and also boost between-island transmission. A 24-h CTV watch is thus required on Martinique and Guadeloupe since they both seem to be virus-free even after *T. citricidus* outbreaks.

insect biology

aphids

• life cycle length

The mean life cycle length of *T. citricidus*, as determined from a sample of 40 apterous aphids, was 17 days (± 2.47) under the environmental conditions of Martinique (Fig. 1).

• breeding rates

The mean number of offspring produced by an apterous female aphid, as determined from a sample of 30 aphids, was 50 aphids (49.6 ±11.3), peaking at almost 60 aphids (59 ± 8) (Table 3).

The first aphids were hatched after 6 days, with maximums at 9-12 days (Fig. 2).

The high standard deviations for these results could be explained by variations in the rearing system.

Nevertheless, the breeding efficiency confirmed field observations, indicating that *T. citricidus* was widely distributed. It seemed to be propagated at the expense of *Toxoptera aurantii*, the main aphid present prior to the arrival of *T. citricidus* but which is now rarely observed.

The high parthenogenic breeding rates of *T. citricidus* enabled it to quickly colonize the West Indies. It has now become the most serious aphid citrus crop pest because of the size of its colonies and the direct crop damage it causes.

The geographic extension of this aphid is also promoted by its marked ability to adapt to various climatic conditions; it is found in wet tropical regions (Asia, South America, Africa south of the Sahara), and in temperate areas (South America, Australia, New Zealand (LECLANT *et al.*, 1992).

parasitoids

• life cycle length

In natural conditions, the mean life cycle length of *L. testaceipes* in the aphid is about 10 days (9.4 ± 1.46), as determined from a sample of 41 insects.

The mean length of the adult *L. testaceipes* life cycle was found to be more than 3 days (3.38 ± 0.9), as determined from a sample of 120 insects placed in rearing boxes under unnatural conditions.

Parasitism rates assessed from field data were too heterogeneous to be interpreted. Nevertheless, it seems that *L. testaceipes* biocontrol could not alone reduce aphid populations to low enough levels to hinder the spread of CTV.

conclusion

The present results indicated that CTV was not yet present in Martinique, despite the arrival of its vector *T. citricidus* at the end of the 1980s. However, further in-depth analyses should now be carried out with more efficient techniques such as immunoprinting, PCR, etc.

Table 2
Citrus production and variation in *T. citricidus* and CTV distributions in the Caribbean Basin (from AUBERT, 1992; CAO VAN, 1992, updated in 1993).

Country	Citrus output	September 1991 CTV MS	September 1991 CTV SS	September 1991 *T. citridus*	September 1992 CTV MS	September 1992 CTV SS	September 1992 *T. citridus*	September 1993 CTV S?	September 1993 *T. citridus*
Costa Rica	34	+	+	+	+	+	+	+	+
Panama	86	+	+	+	+	+	+	+	+
Nicaragua	66	+	+	+	+	+	+	+	+
El Salvador	99	+	–	–	+	?	–	+	–
Honduras	97	–	–	–	+	+	–	+	–
Guatemala	135	–	–	–	+	–	–	+	–
Belize	87	+	–	–	+	+	–	+	–
Mexico	3 081	–	–	–	–	–	–	–	–
Trin. & Tobago	16	–	–	–	+	+	+	+	+
Saint Lucia	–	–	–	–	?	?	+	+	+
Martinique	2	–	–	–	–	–	+	–	+
Dominica	20	–	–	–	?	?	?	?	+
Guadeloupe	4	–	–	–	–	–	+	–	+
Puerto Rico	33	–	–	–	+	+	+	+	+
Dominican Rep.	84	–	–	–	+	+	+	+	+
Jamaica	104	–	–	–	+	?	+	+	+
Cuba	898	–	–	–	(–)	(–)	+	?	+
Grenada	2							–	+
Saint Vincent	1							–	+
Antigua	–							+	+
Nevis	–							–	+
Saint Kitts	–							–	+

CTV = citrus tristeza; MS = mild strain; SS = severe strain; S? = severity unknown.

Figure 1
Length of the T. citricidus *life cycle.*

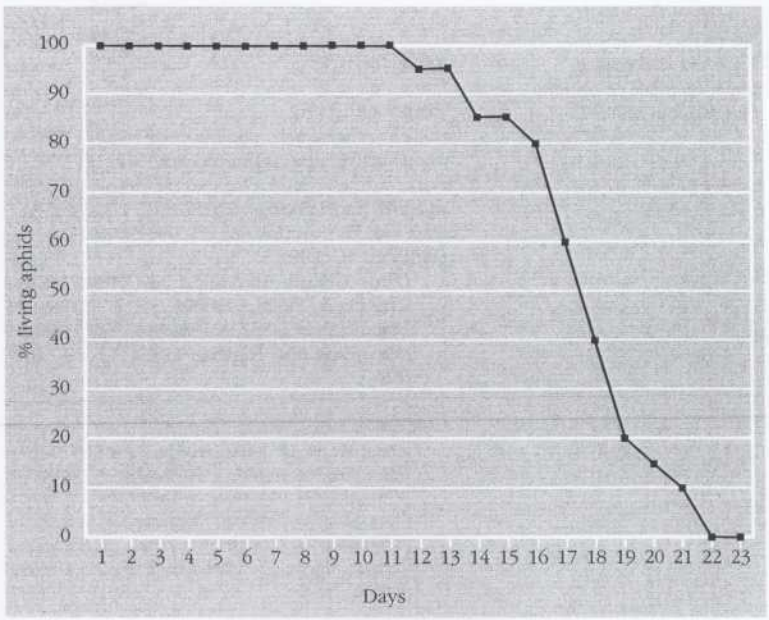

Table 3
Cumulated offspring for an apterous *T. citricidus* female.
The results were calculated from the offspring of 30 female aphids.

Life cycle length (days)	Total number of hatched aphids	
12	34.7	(8.44)*
13	40	(9.17)
14	45.5	(9.75)
15	49.6	(8.25)
16	53.2	(7.94)
17	57.6	(6.18)
18	58.2	(5.76)
19	59	(8)

* (x) = standard deviation

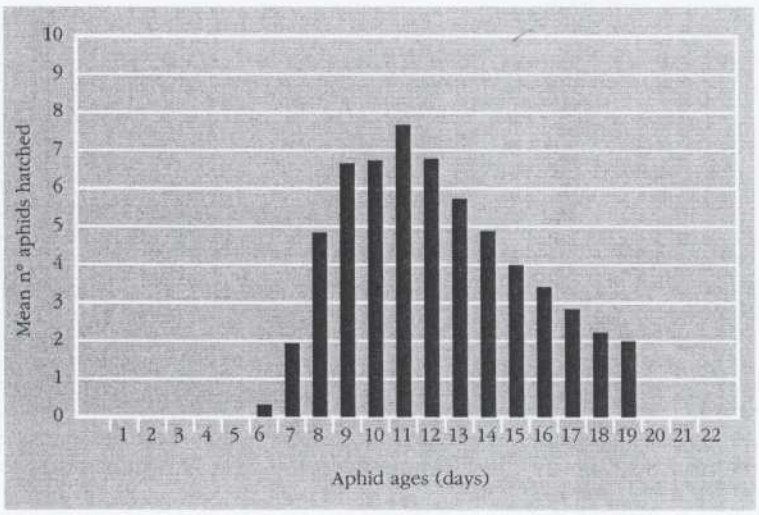

Figure 2
T. citricidus *breeding (mean number of aphids hatched/day/parent).*

references

AUBERT B., 1992.
Le programme agrumes du CIRAD-FLHOR. *Fruits*, Special issue on *Citrus* 47: 99-102.

AUBERT B., ETIENNE J., COTTIN R., LECLANT F., CAO VAN Ph., VUILLAUME C., JARAMILLO C., BARBEAU G., 1992.
Citrus Tristeza disease, a new threat for the Caribbean Basin. Report of a survey to Colombia, Dominican Republic, Guadeloupe, Martinique and Trinidad. Fruits 47 (3): 393-404.

CAMINADE J.M., 1992.
Le virus de la Tristeza des agrumes (CTV): transmission par *Toxoptera citricidus* (Kirkaldy) (*Hemipterae: Aphididae*) en conditions semi-contrôlées. Facteurs influençant la concentration des plantes hôtes en parasites viraux. Thesis. Cergy-Pontoise, France, ISTOM, 77 p.

CAO VAN P., 1992.
Citrus Tristeza virus/*Toxoptera citricidus*: situation in Martinique, Guadeloupe (French West Indies) and St Lucia. *In*: CTV and *T. citricidus* in Central America. Development of management strategies and use of biotechnology for control, September 14-19 1992, Maracay, Venezuela, 287 p. CATIE (Costa Rica), University of Florida (USA), INIFAP-SARH (Mexico), Universidad Central de Venezuela, USDA (USA), p. 102.

GILLETT J.M., MORRISSEY S.M., RAMSDELL D.C., 1986.
Interpreting ELISA data and establishing the positive-negative threshold. Plant Disease 70 (8): 722-726.

LECLANT F., ETIENNE J., AUBERT B., 1992.
Alerte à la Tristeza en verger d'agrumes. Le puceron vecteur *Toxoptera citricidus* envahit l'arc Caraïbe. Phytoma (440): 32-34.

ROISTACHER C.N., GUMPF D.G., DODDS J.A., LEE R.F., 1991.
The threat of «the citrus killer». Citrograph 76 (10): 4-10.

STARY P., LYON J.P., LECLANT F., 1988a.
Post-colonisation host range of *Lysiphlebus testaceipes* in the Mediterranean area (*Hymenoptera, Aphidiidae*). Acta Entomol. Bohemoslov. 85: 1-11.

STARY P., LYON J.P., LECLANT F., 1988b.
Biocontrol of aphids by the introduced *Lysiphlebus testaceipes* (Cress.) (*Hymenoptera, Aphidiidae*) in Mediterranean France. J. Appl. Ent. 105 : 74-87.

WALLACE J.M., 1978.
The Tristeza disease complex. The citrus industry. University of California, USA. W. Reuther, E.C. Calavan and G.E. Carman, eds, Volume IV, p. 87-109.

YOKOMI R.K., 1992.
Potential for biological control of *Toxoptera citricidus* (Kirkaldy). *In*: CTV and *T. citricidus* in Central America. Development of management strategies and use of biotechnology for control, September 14-19 1992, Maracay, Venezuela, 287 p. CATIE (Costa Rica), University of Florida (USA), INIFAP-SARH (Mexico), Universidad Central de Venezuela, USDA (USA), p. 194-198.